



TITLE:

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CITATION:

MATSUO, YASUO. Morphological Studies on the Mice Livers Administered Repeatedly with Impurities-Enriched Halothane. 日本外科宝函 1972, 41(2): 100-118

ISSUE DATE:

1972-11-01

URL:

<http://hdl.handle.net/2433/207951>

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Morphological Studies on the Mice Livers Administered Repeatedly with Impurities-Enriched Halothane

by

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Received for Publication Nov. 29, 1971

Introduction

Against the old general concept that halothane has minimum specific toxicity upon the liver, several hepato-toxicological studies have been carried out in order to clarify the potential hazard not only of commercial halothane itself but also of its impurities.^{1-10,13)}

There are two types of reaction for synthesis of halothane, i.e., high thermal reaction and low thermal reaction. During the past years a number of gas chromatographic analysis of anesthetic halothane revealed the presence of several different impurities in two different 'halothanes'.^{14,15)} However, the major impurity in the halothane produced by high thermal synthesis is dichlorohexafluorobutene (DCHFb), and the one manufactured by low thermal reaction is dichlorohexafluorocyclobutane (DCHFcb) which toxicity was considered to be unimportant by SCHERER and OEHMIG.^{6,8)}

In 1963 COHEN suggested the possible role of DCHFb in anesthetic halothane as a toxic factor,¹⁾ and reported that the concentration of this impurity tended to increase in the residue under the presence of copper and oxygen. In the same year SEXTON and HENDRICKSON,²⁾ later in 1965 PHILLIPS and his co-workers,⁵⁾ indicated that the copper was of lesser importance for increasing the concentration of the impurity than the concentration effect produced by evaporation.

Several observers have suggested that commercial halothane produces a lesion in human liver similar to that associated with fulminating viral hepatitis, and it has also been pointed out that typically the hepatic necrosis which has been attributed to anesthetic halothane has not occurred after a single exposure, but after a second or subsequent exposures to the agent following a rest period.¹⁰⁻¹²⁾ This fact suggests that the injury may be the result of sensitization of the liver cell to commercial halothane or to halothane-impurities in it.

The following study represents morphological investigation of the hepatotoxicity of halothane, DCHFB and DCHFCB, testing the effect of impurity-enriched halothane on the liver of mice in repeated administration.

Material and methods

One hundred sixty D. D. strain mice of both sexes weighing 17 to 22 gm were subjected to the study divided into seven groups according to the kind of impurity and its concentration.

Table 1

Group A.	1 % halothane itself without added impurity.	20 mice.
Group B.	1 % halothane enriched with 50 p. p. m. DCHFCB.	20 mice.
Group C.	1 % halothane enriched with 100 p. p. m. DCHFCB.	20 mice.
Group D.	1 % halothane enriched with 250 p. p. m. DCHFCB.	30 mice.
Group E.	1 % halothane enriched with 50 p. p. m. DCHFB.	20 mice.
Group F.	1 % halothane enriched with 100 p. p. m. DCHFB.	30 mice.
Control.		20 mice.

Cylindrical glass chamber, 8-liters in capacity, was prepared for each group to anesthetize them with 1 % halothane vapor enriched with 50, 100, 250 p. p. m. (v/v.) DCHFCB or 50, 100 p. p. m. DCHFB respectively. Control animals were simultaneously maintained in an identical cage in room air. The total number of animals in each group is listed in table 1.

Vaporization of the agent was done with 5 liters per minute pure oxygen of flow through Fluotec vaporizers. Cups of water were placed in the chambers to keep moderate humidity, and attention was also paid to keep surrounding temperature around 24 degree C. constantly. All animals were, of course, allowed standard meal and water ad libitum.

One hour exposures to the agents were repeatedly given to each experimental group daily for ten consecutive days. At the termination of 'anesthesia' on the tenth day the half of the subjects were sacrificed by means of decapitation and the liver specimens were immediately taken out from the central portion of the larger right lobe. Animals of the other half were maintained without any anesthesia for the following ten days as 'Recovery Period', and then sacrificed.

For electron microscopy small pieces of liver tissue were fixed for 90 minutes in a cold 1 per cent solution of phosphate-buffered osmium tetroxide (pH 7.4), dehydrated with alcohol in ascending concentrations, infiltrated and embedded in Epon 812.¹⁶⁾ They were sectioned on a Nippon-Denshi JUM-5 A microtome with glass knives, stained with lead citrate or lead hydroxide,¹⁷⁾ and examined with a Hitachi HU-11 A model electron microscope.

For light microscopic observation, routine sections of formalin-fixed tissue which were embedded in paraffin and stained with hematoxylin-eosin and Sudan III were also studied.

Normal mouse liver

The parenchymal cell appeared polygonal or flat rhombic in shape.

The plasma membrane or cell membrane took the form of so-called "unit membrane",¹⁸⁾ which consisted of three layers, outer two layers being of relatively high density and inner one of lower density. Two adjacent cell membranes were in close contact with each other, and the interspace between them was about 150 Å in width, but in some places they formed sinusoids and spaces of DISSE, where microvilli protruded from the membranes.

Each cell contained one or two nuclei which were usually elliptic in shape, and one to three dense glomerular nucleoli existed in a nucleus without any membranous structures. The nuclear membrane or nuclear envelope¹⁹⁾ consisted of two layers.

Mitochondria were generally round or elliptic. However, they occasionally appeared multiple in shape, e. g. butterfly- or kidney-shaped. Cristae mitochondriales and mitochondrial membranes^{20,21)} also consisted of three unit membranes and intramitochondrial granules are found in mitochondrial matrices which were slightly darker than surrounding cytoplasmic matrices.

Endoplasmic reticulum was found most abundantly around mitochondria, and liposomes were usually located within granular or ggranular reticulum. Geometrical arrangement of ribosomes and their association with cisternae of endoplasmic reticulum was frequently observed.²²⁾

GOLGI apparatus was seen in the vicinity of nucleus or in peripheral cytoplasm near capillary bile duct, and it consisted of GOLGI cisternae and GOLGI vesicles.²³⁾

Aggregation of dark-staining glycogen granules was easily found when stained lead citrate or lead hydroxide.

Fat droplets were occasionally observed by electron microscopy even if they were negative by Sudan III stain. However, the identification or differentiation from other osmiophilic round figures was sometimes very difficult.

Lysosomes,²⁴⁾ approximately 0.4-1.0 micron in diameter, appeared round or elliptic in shape, and so did microbody.

Changes in the livers of mice

A.) Mice anesthetized with 1 % halothane itself without added impurities.

Under light microscopic observation, marked fatty infiltration was seen in the centrilobular areas by SUDAN III stain, as throughout found in every experimental group in the series of this study. Any other changes such as cellular degeneration and necrosis could not be found out.

Electron microscopically a decrease in the amount of glycogen was observed, and small electron opaque bodies or liposomes were found in the most of the parenchymal cell cytoplasm, particularly within the endoplasmic reticulum, although they were present even in the control animals in a small number. Giant liposomes which could be seen to derive from coalescence of the smaller ones were also found occasionally. The cavities of the endoplasmic reticulum were slightly dilated, and the ribosomes

were somewhat dispersed, subsequently minimal increase in the number of smooth vesicles was observed. Microbodies were increased in number. The mitochondria were sometimes bizarre in shape, but in general they appeared almost normal. In some areas very light and very dense cells were seen adjacent to one another. The bile canaliculi, the sinusoidal lining cells and the KUPFFER cells were normal.

B.) Mice administered with 50 p. p. m. DCHFCB in 1 % halothane.

Changes not greater than in the group A. (anesthetized without the impurities) were demonstrated in the hepatocytes.

C.) Mice administered with 100 p. p. m. DCHFCB in 1 % halothane.

Beside the central fatty infiltration by SUDAN III stain, mild focal necroses were occasionally seen in the specimens stained with hematoxylin and eosin. The extent of the necrosis was limited, but the lesions were somewhat numerous when compared with those of the mice anesthetized with 100 p. p. m. DCHFB in 1 % halothane vapor (Group F.), which will be stated later in this paper. However, electron microscopic observation revealed that only a few minimal pathologic changes were found in the overall cell structure. Unfortunately the deteriorated hepatocytes suggesting the lesions of light-microscopical necroses could not be observed under electron microscopy in spite of repeated trial to find out from each specimen in this group. This fact means that each lesion of the focal necrosis was small. The decrease in glycogen areas and the appearance of numerous small electron opaque bodies, probably fat droplets, were found in the cytoplasm. Some mitochondria were slightly swollen and of odd shape, but scarcely showed the crenation of mitochondrial membrane. The density of mitochondrial matrix appeared to be the same as in control animals. Lysosomes showed a small increase in number; and so did the smooth surfaced endoplasmic reticulum, thereby slight dispersion of ribosomes was observed.

D.) Mice administered repeatedly with 250 p. p. m. DCHFCB in 1 % halothane vapor.

The severity of miliary necrosis was not so masked as those administered 100 p. p. m. DCHFCB in 1 % halothane. Fatty infiltration in the centrilobular area was, demonstrated under light microscopy, as was in all of the other experimental groups of mice in this work.

Electron microscopically, several finer structural alterations were found in the hepatocytes. Mitochondria was not uniform in size and shape, videlicet, many of mitochondria were elongated or enlarged, having bizarre shape with increased number of cristae of increased length. In the same hepatocytes there coexisted numerous degenerating mitochondria. Some of them showed decrease in the numbers of cristae and their own sizes. Some others, of which individual mitochondrial membrane was partly or completely unrecognizable, preserved their cristae well. Crenation of mitochondrial membrane was occasionally seen. The density of mitochondrial matrix appeared almost normal, and the dense intramitochondrial granules were not increased in number despite the larger size of the organelles.

Autophagic vacuoles were often found in cytoplasm, and most of them contained either single mitochondrion or endoplasmic reticulum.

The profiles of endoplasmic reticulum showed slight dilatation and a small increase in the number of smooth surfaced reticulum. In some area the ribosomes were dispersed and clustered mostly near the membrane of the endoplasmic reticulum. However, the majority of the ribosomes remained attached to the endoplasmic reticulum.

A large number of liposomes appeared near or within the endoplasmic reticulum, and fusion of liposomes and giant liposomes were also indicated.

GOLGI complex appeared somewhat enlarged and usually contained osmiophilic bodies whose relationship to the other dense bodies such as liposomes was obscure. The remainder of the cell structure, such as nuclei, bile canaliculi and sinusoidal spaces of DISSE, appeared almost normal.

E.) Mice administered repeatedly with 50 p. p. m. DCHFB in 1 % halothane vapor.

Spotty lesions of focal necrosis were occasionally seen by light microscopy. They were observed typically in one fourth of the subjects and, when present, they were mainly located in the peripheral region of the lobules. The necrotic lesions in this experimental group were much less numerous than those in DCHFCB group although they were much more sequestered. The liver cell nuclei in the vicinity of the necrotic area tended to be swollen or pyknotic. Fatty infiltration was also observed in all specimen.

In electron micrograph mitochondrial shrinkage was frequently seen. Such mitochondria was usually round or oval in shape, while others were shaped irregularly. The cristae of them appeared a little shortened and somewhat decreased in number, while the crenation of mitochondrial membranes was frequently observed here and there. However, the mitochondrial matrices kept their density to be within the normal limits and the intramitochondrial granules, although present in the control animals, appeared less numerous. The rest of the mitochondria remained normal in appearance with normal cristae.

Small myelin figures, 0.2 to 0.3 micron in diameter, were occasionally to be attached to or within the mitochondria. The mitochondria with such figures were rather normal in size than those without.

The endoplasmic reticulum showed an increase in the number of smooth vesicles, and aggregation of many polyribosomes were clustered usually in the perinuclear zone, on or very near the profiles of endoplasmic reticulum. Liposomes, especially giant liposomes were much increased in size and number. These liposomes were observed in association with the dilated endoplasmic reticulum or within vacuoles of the GOLGI complex.

The bile canaliculi and pericanalicular ectoplasm were normal, as were the sinusoidal microvilli.

F.) Mice administered repeatedly with 100 p. p. m. DCHFB in 1 % halothane vapor.

No remarkable difference between the hepatic changes of the group E. and F.

was found under light microscopical observation.

Ultrastructurally, the following changes could be found in this group. The crenation of mitochondrial membranes was more frequently seen, and the number and length of the cristae mitochondriales were reduced in some areas. Furthermore, the myelin figures were predominantly found in the hepatocytes, most of which were located characteristically either on the edge of the mitochondrion or in between adjacent mitochondria. However, these myelin figures were observed in the half of the animals examined and, when present, were in each block studied but not always in each cell.

The endoplasmic reticulum was dilated, shattered in some areas, and was transformed into smooth vesicles in other areas. They became much extensive and usually did not form whorls. The ribosomes were dispersed in the hyaloplasm and some single ribosomes or shorter coils remained attached to the endoplasmic reticulum. The dispersion of ribosomes did not seem to be associated with an appreciable alteration of the endoplasmic reticulum.

The giant liposomes, spherical or elliptic in shape, were increased in size, often 4.0-4.5 microns in diameter, and still maintained moderate electron opacity in general. As these giant liposomes became strikingly bigger, they subsequently used to push some mitochondria and reticulum aside.

Autophagic vacuoles, or lysosomes, were increased in number when compared to control mice or to the other experimental group in this work.

Occasional light and dense cells were seen. The other structure, including the bile canaliculi and the sinusoidal spaces of DISSE, appeared almost normal.

Recovery. (10 days after the last exposure.)

In any group of the series of this experiment the focal necrosis was no more found under light microscopy, instead a few small areas presumably of connective tissue were scattered in some specimens, suggesting that these wounded areas were on the way of healing in this phase. Moreover, the fatty infiltration was almost faded away from the lobules.

Even in the electron microscopic observation the size and number of the fatty droplets, liposomes and giant liposomes, were considerably reduced. So were the degenerating mitochondria. Although some of the mitochondria of the group D. and F. were still of odd shape, the hepatocytes in general seemed to have recovered almost to normal. A few autophagic vacuoles, slight glycogen depletion and somewhat enlarged GOLGI apparatus were found in some cells, but this was not uniform and changes in any single cell were not so severe as at the termination of the tenth exposure to each agent. The myelin figures were scarcely seen. In addition, the disaggregation and dispersal of surface ribosomes were corrected so that reconstitution of the ribosomal coils was observed in all specimen.

The bile canaliculi and sinusoidal microvilli appeared quite normal.

Discussion

The presence of DCHFB in commercial halothane has been considered to present a potential hazard,⁷⁾ and that of DCHFCB to have a minor toxicity.^{6,8)} In fact, in our laboratory, DCHFB of approximately 250 p. p. m. in oxygen for one hour exposure induced diarrhea and nervous system irritation, and at last. These mice in most cases died following spastic convulsions even by minimum stimulation such as touching their bodies softly.

The initial change found in the livers of the mice in "halothane alone" group was, as previously described, the central fatty infiltration which, however, was also seen when 0.5% metoxyflurane was administered repeatedly in the same fashion as this experiment.²⁵⁾

The fatty infiltration, that is, the engorgement of the endoplasmic reticulum by the electron opaque bodies or liposomes under ultrastructural observation could be considered to be the earliest morphological expression of the interference in lipoprotein metabolism,^{28,35)} since the basis for the lipid accumulation is presumably a block in the synthesis of lipoprotein by the liver or in the transfer of this plasma protein to the blood.²⁹⁾

As COHEN reported,⁷⁾ major halothane impurity does undergo metabolism within the liver. The role of the liver in detoxification of the impurities has been demonstrated through studies of hepatic clearance and by activation analysis techniques.⁷⁾ In view of this fact the administration of DCHFB- or DCHFCB-enriched halothane may contribute some potential hazards to hepatocytes. The changes such as focal necrosis, mitochondrial transformation and appearance of mitochondrial myelin figures do give a considerable suggestion to this problem. Focal necrosis in the liver of DCHFB group resembles that in the liver of a mouse suffering from typhus, though none of the experimental animal seemed to have an attack of the disease of such kind. In DCHFCB group, the grade of millary necrosis is slighter and the extent of necrosis is limited, but the lesions are more numerous when compared with the livers of DCHFB group. The differences between the natures of two types of focal necroses and the presence of mitochondrial myelin figures in one of the groups might be considered to have derived from the pharmacological differences of these two impurities. Even the mitochondrial alterations in each group, as stated above, take the different types respectively.

The observation that the mitochondria appear to undergo a turnover after ten day's repeated administration of the impurities in higher concentration, as evidenced by the formation of many lysosomes or autophagic vacuoles, could be led to the idea that the impurities make the lysosomal membrane more permeable and lead to leakage of hydrolytic enzymes from the lysosomes, which might result in cell damage.³⁰⁾ The damaged organelles, e. g. mitochondria and endoplasmic reticulum, are, however, sequestered in the lysosomes, and the most of remaining cell structures are almost normal in appearance rather than deteriorated. Therefore, it is plausible that the impurities exert direct effects upon mitochondria and endoplasmic reticulum. Nevertheless, the question whether these effects should be classified as toxic or non-

Table 2. Weights of Mice of Group D. *

* Mice were administered with 250 p. p. m. DCHFCB in 1 % halothane vapor for one hour daily on ten consecutive days.

(15 mice were selected at random.)

Weight	Before 1st exposure	After 5th exposure	After 10th exposure	After rest period
Mouse 1	19	17	18	sacrificed after exposure
2	20	19	18	sacrificed after exposure
3	20	16	17	sacrificed after exposure
4	18	17	16.5	sacrificed after exposure
5	18	15	16.5	sacrificed after exposure
6	20	18	16	sacrificed after exposure
7	19	14	13	18
8	22	20	16.5	22
9	19	16	16	18
10	20	18	19	22
11	18	15	14	20
12	19	16	15	dead
13	18	16	14	dead
14	21	22	20	22
15	18	15	14	dead
Average	19.3	17.1	16.2	20.3

Table 3. Weights of Mice of Group F. *

* Mice were administered with 100 p. p. m. DCHFB in 1 % halothane vapor for one hour daily on ten consecutive days.

(15 mice were selected at random.)

Weight	Before 1st exposure	After 5th exposure	After 10th exposure	After rest period
Mouse 1	17	14	dead	
2	20	17	dead	
3	18	16	16	sacrificed after exposure
4	18	15	14	sacrificed after exposure
5	18	14	dead	
6	18	16	17	sacrificed after exposure
7	18	16	dead	
8	18	16	17.5	sacrificed after exposure
9	19	16	17	sacrificed after exposure
10	20	17	19	22
11	18	16	17	19
12	19	18	19	20
13	16	15	16	dead
14	18	18	16	17
15	18	17	14	dead
Average	18.2	16.1	17.5	19.5

toxic remains unanswered. For reference, the animal continued to lose weight during the period of administration. (Table 2. 3.)

One per cent halothane vapor in pure oxygen means hyperoxic condition, under which the blood oxygen is elevated and, as KATCHMAN reported,³¹⁾ the hepatic oxygen consumption in vitro is increased above normal. This suggests a change in the NADH/NAD ratio with formation of excess NAD, a situation which is the reverse of that found in hypoxia.³²⁾ Now the liver seems sensitive to metabolic changes, since the animals breathing pure oxygen for prolonged period often show some ultrastructural cellular changes in hepatocytes such as glycogen depletion, mitochondrial transformation with bizarre shapes and appearance of small myelin figures,³⁰⁾ which were also observed in this study. These myelin figures, as TRUMP stated,³³⁾ can be produced even by freezing and thawing of the liver after it has been excised. None of these changes, therefore, can be regarded as specific for halothane impurities.

Recently the content of the both impurities in currently available halothane has been tremendously reduced as well as several other minor impurities.³⁴⁾ Yet the complete removal of such contaminants from anesthetic halothane is nowadays almost impossible. And so, for the sake of convenience in this study, the halothane prepared by low thermal synthesis has been used as "halothane alone".

On gas chromatogram only a small notch of less than 20 p. p. m. of the major impurity is detectable in today's commercial halothane,^{15,34)} which is amazingly compared with high peaks of such contaminants in old brand halothane.

Therefore, halothane in our laboratories could be considered less hepatotoxic or conveniently no more problem in liver toxication in ordinary use. However, the extension of these observations in mice to primates, particularly human being, must yet be studied before these impurities even in lower concentration can be considered innocuous for men, and for more and more safety the complete removal of the impurities from currently available halothane should be carefully thought out.

Summary

The livers of mice exposed to impurity-enriched 1 % halothane for 1 hour daily on ten consecutive days were examined light and electron-microscopically in order to study hepatotoxic effects of both DCHFB and DCHFCB.

Halothane, even if it contains DCHFCB in lower concentration of 50 p. p. m., cause glycogen depletion and centrilobular fatty infiltration, so that the appearance of many liposomes and giant liposomes near slightly dilated endoplasmic reticulum is observed in most of hepatocytes. In addition to these alterations, numerous lysosomes and mitochondria of odd shapes appear when the concentration of DEHFCB is elevated to 250 p. p. m.. These changes persist also in the case of 50 p. p. m. DCHFB, and occasional mitochondrial myelin figures are found in some specimens. Mitochondrial alterations with the crenation of mitochondrial membranes are also seen. All of the changes are emphasized as the concentration of DCHFB is doubled up to 100 p. p. m., but at 250 p. p. m. the mice scarcely survive the experiment. Each impurity may cause the focal necroses of different types respectively.

After ten days of rest period, the liver cells are found on the way of restoration in any case. The changes may or may not be specific for the impurities, but at least can be considered as the result of toxicities of these impurities. Therefore, the complete removal of the impurities from halothane should not be forgotten, though their concentrations nowadays are tremendously reduced.

Acknowledgement

Grateful acknowledgement is made to Prof. AKIRA INAMOTO, Dept. of Anesthesiology, Kyoto University, for his invaluable advice and encouragement in various phases of this work.

Acknowledgement is also extended to Prof. KOZO OKAMOTO, Dept. of Pathology, Kyoto University, and Prof. FUMIHIRO ICHIDA, Dept. of Internal Medicine, Niigata University, for their helpful suggestions and criticisms, to Dr. TAKESHI OKAMOTO and Miss MARIA YATSUI for their able technical assistance, and finally to Mr. SADAU UCHIDA and Mr. MAKIO FUJIOKA for kind advice and assistance in the photography part of this work.

The halothane and both of its major impurities were generously prepared by Daikin Industry Co. Ltd.

This study was supported in part by research grants from the Ministry of Education, Grant No. 721530 & 721670.

The paper was partly presented at the 2nd Asian & Australasian Congress of Anesthesiology, Sept. 1966, at the 15th Congress of Japan Society of Anesthesiologists, Apr. 1968, and at the 4th World Congress of Anesthesiologists, Sept. 1968.

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Key to figures	A, autophagic vacuoles	Lp, liposomes
	ar, agranular reticulum	Ly, lysosomes
	C, bile canaliculus	M, mitochondria
	ER, endoplasmic reticulum	Mc, microbodies
	GL, giant liposomes	N, nuclei
	G, Golgi apparatus	S, sinusoids

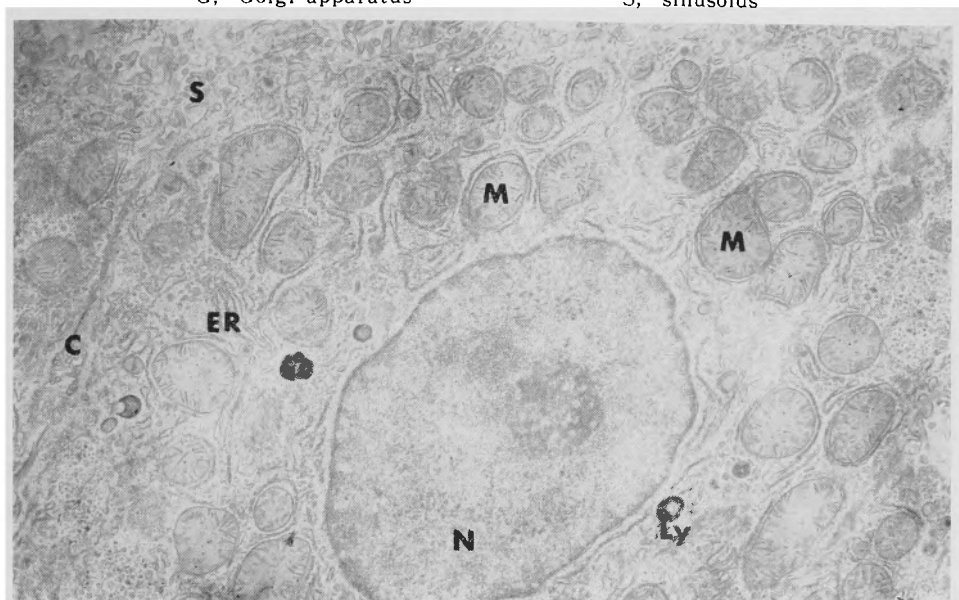


Fig. 1 Electron micrograph of hepatocyte of control mouse. X 8,000

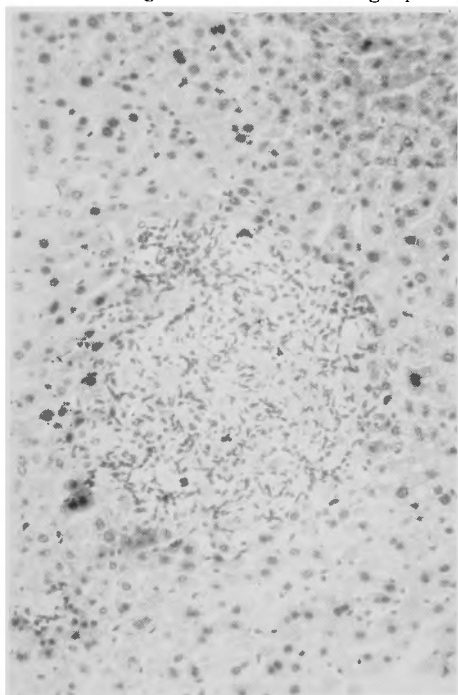


Fig. 2 Necrotic lesion is located in the peripheral region of the lobule, and more sequestered than Fig. 3. Hematoxylin and eosin. 100 p. p. m. DCHFB.

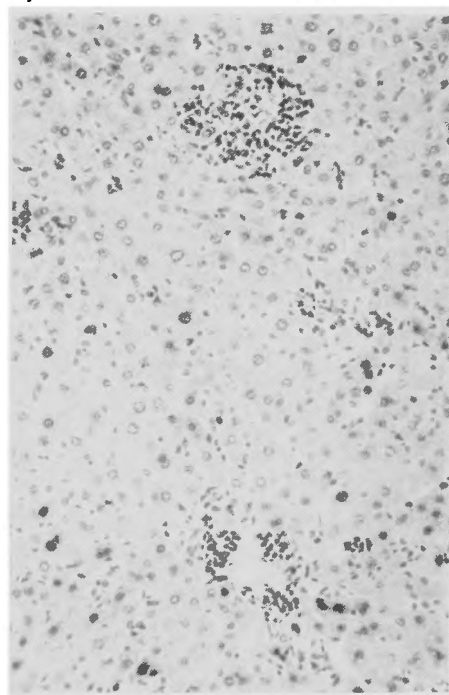


Fig. 3 Slight focal necrosis is seen. The extent of the necrosis is limited. Stained with hematoxylin and eosin. 100 p. p. m. DCHFCB.

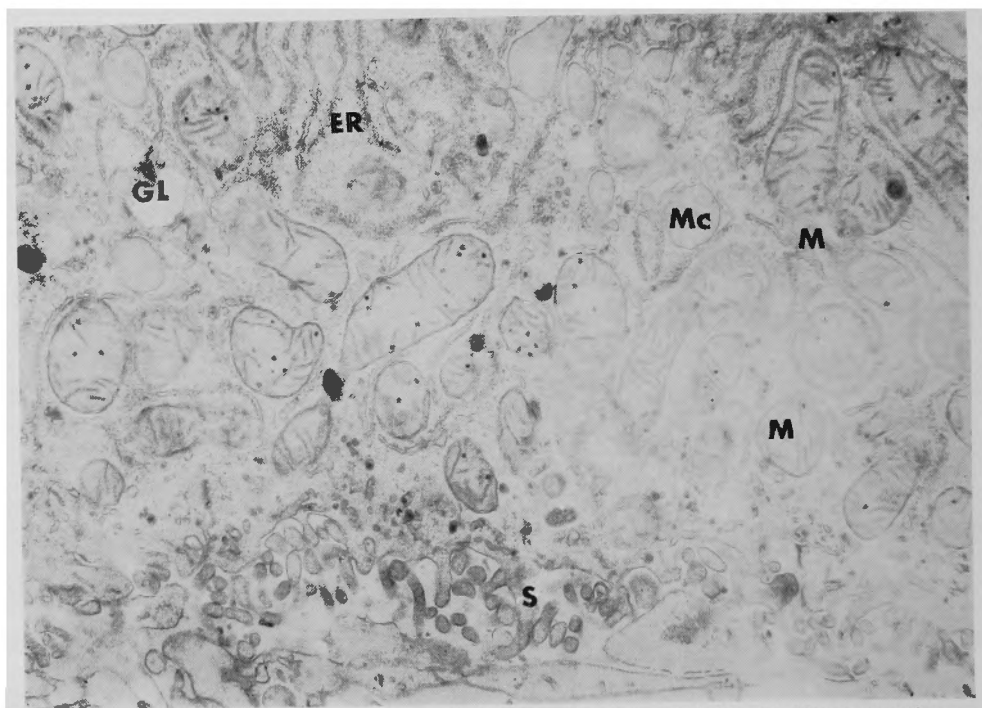


Fig. 4 Hepatocytes exposed to 1% halothane for 1 hour daily on 10 consecutive days. X 10,500

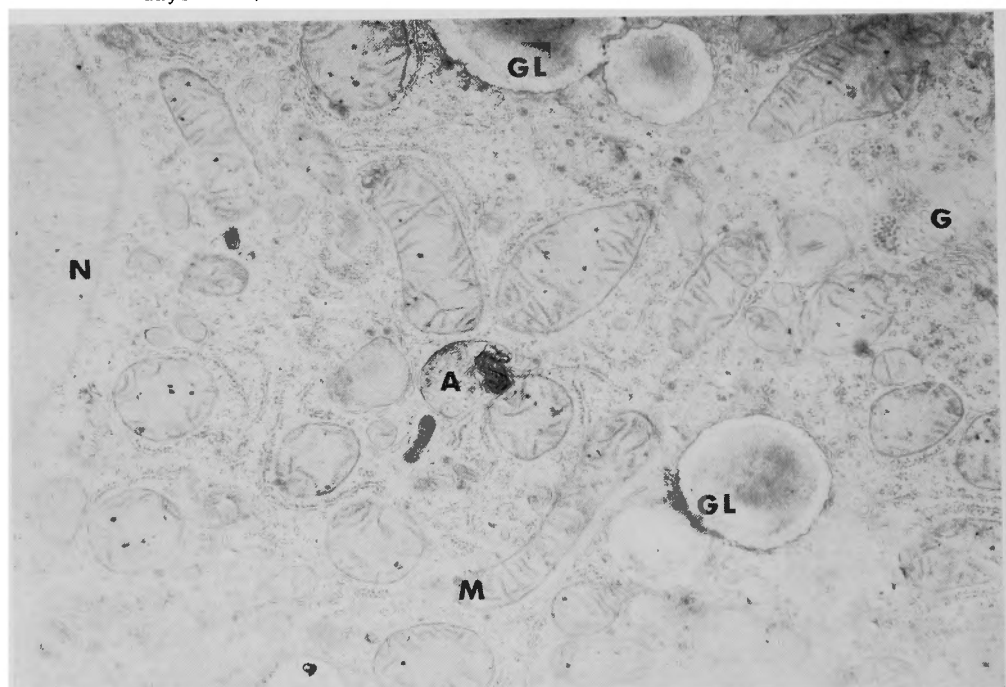


Fig. 5 Portion of hepatocyte administered with 50 p.p.m. DCHFCB in 1% halothane for 1 hour daily on 10 consecutive days. Fusion of two adjacent giant liposomes is seen. X 16,000

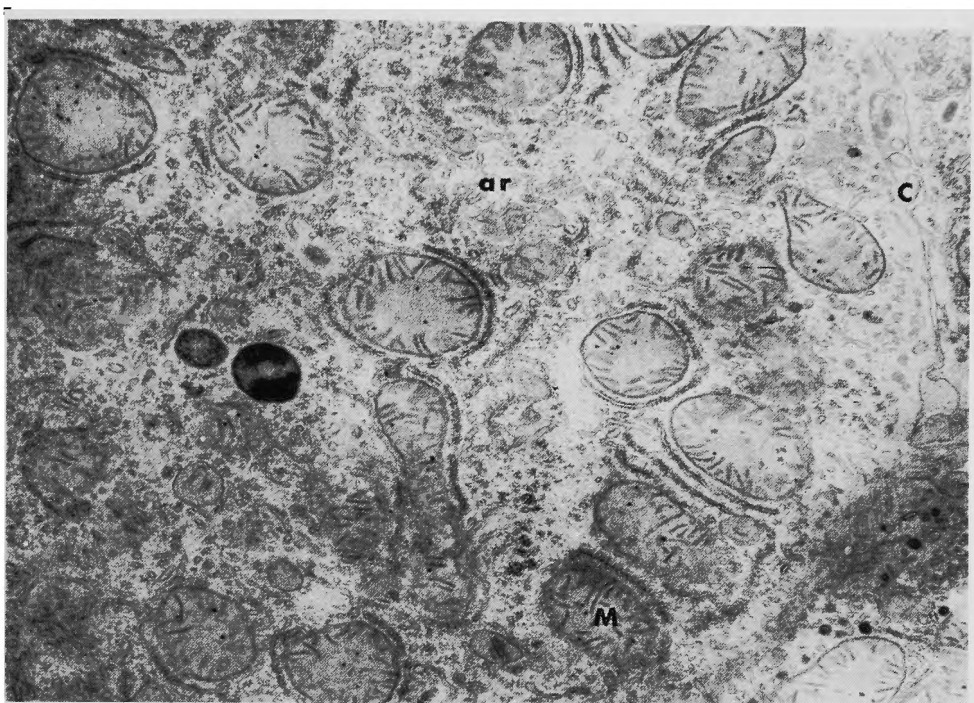


Fig. 6 100 p. p. m. DCHFCB is added to 1% halothane vapor. The endoplasmic reticulum shows a slight increase in the number of smooth vesicles. X 16,000

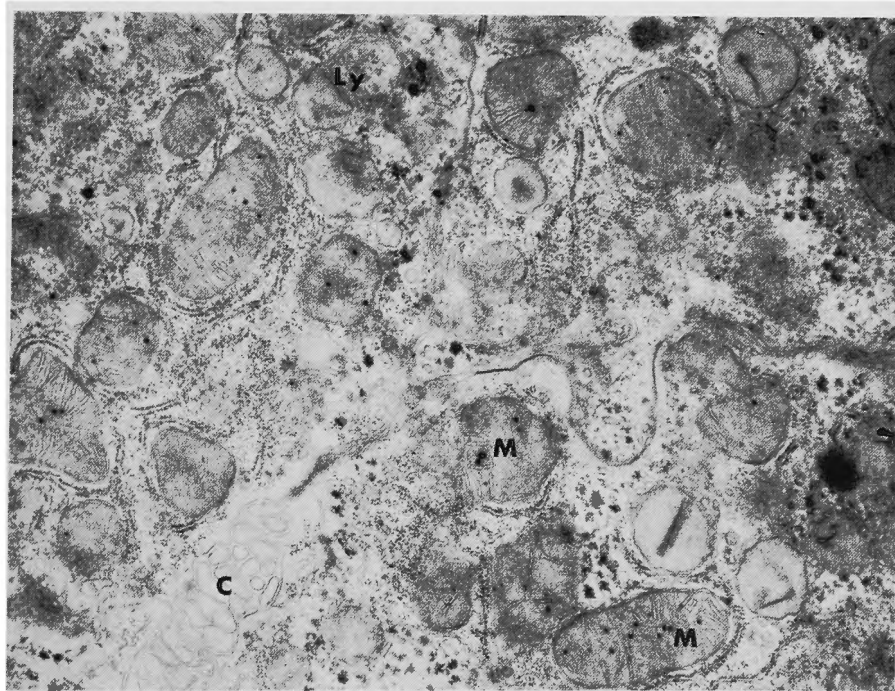


Fig. 7 Portion of three hepatocytes exposed to 1% halothane containing 250 p. p. m. DCHFCB for 1 hour daily on 10 consecutive days. X 16,000 Slight crenation of mitochondrial membrane is seen in lower cell.

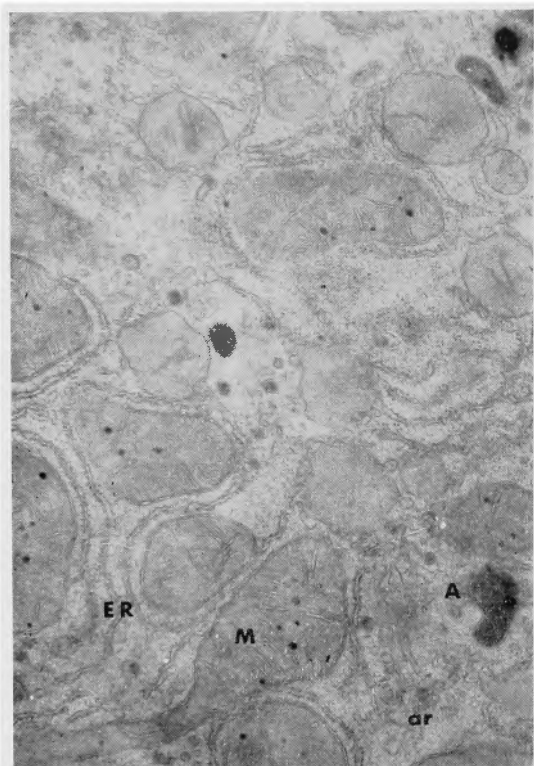


Fig. 8 A large, elongated mitochondrion with the crenation of mitochondrial membrane and an autophagic vacuole are seen. DCHFBCB of 250 p. p. m. is enriched in 1% halothane. Immediately after 10th exposure. X 27,000

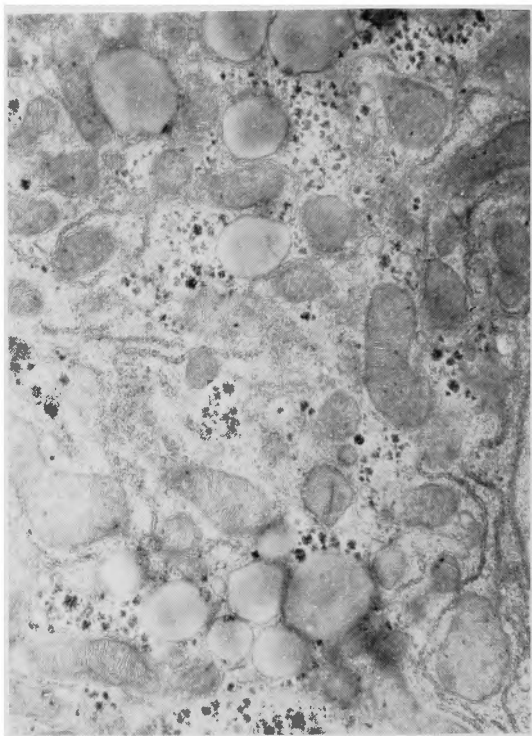


Fig. 9 Portion of liver cell administered with 250 p. p. m. DCHFBCB-enriched halothane for 1 hour a day on ten consecutive days. Note liposomes and many giant liposomes within or near the endoplasmic reticulum. X 16,000

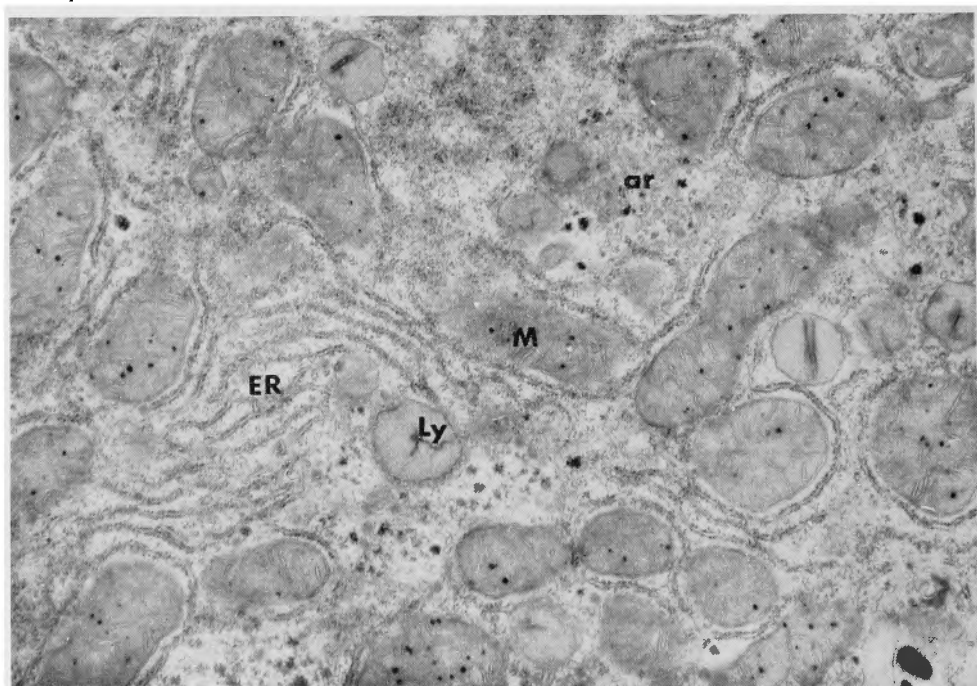


Fig. 10 The ribosomes are dispersed in the hyaloplasm. 250 p. p. m. DCHFBCB. X19,000

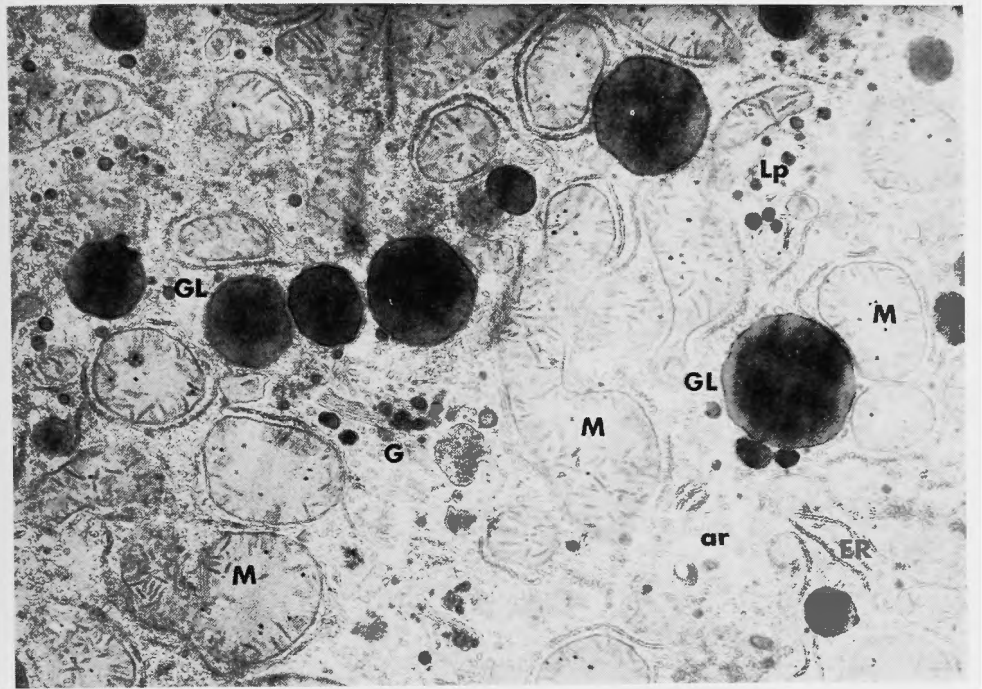


Fig. 11 Fusion of liposomes and giant liposomes is indicated. Mitochondrion of odd shape is seen. 50 p. p. m. of DCHF B is added to 1 % halothane, and daily 1 hour exposure is repeatedly performed for 10 days. X 19,000

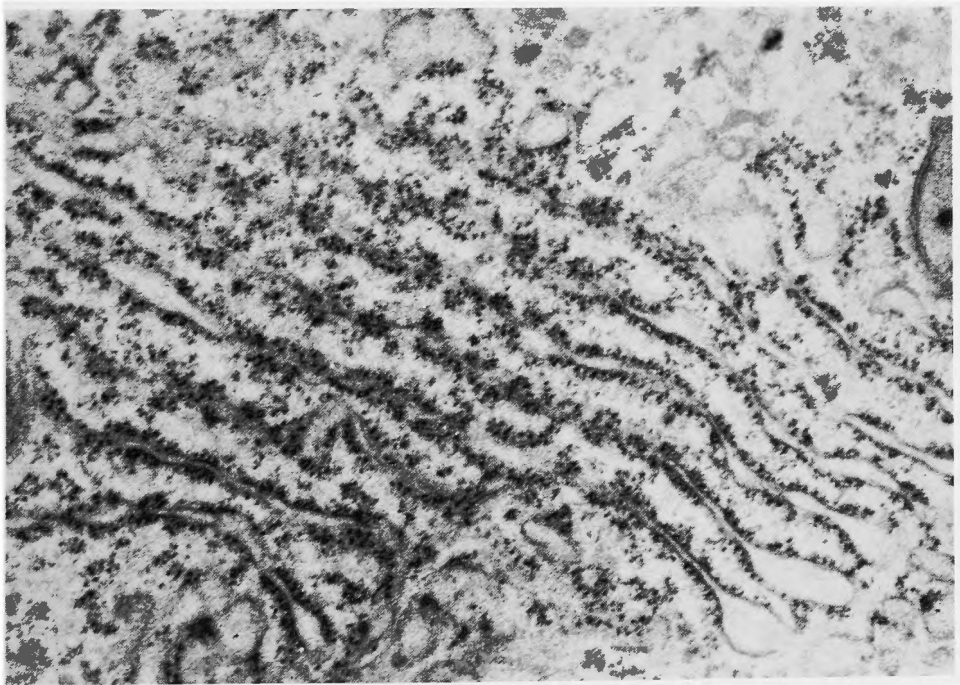


Fig. 12 Ribosomes are irregularly arranged. Higher magnification of the same specimen as Fig. 11. X 56,000

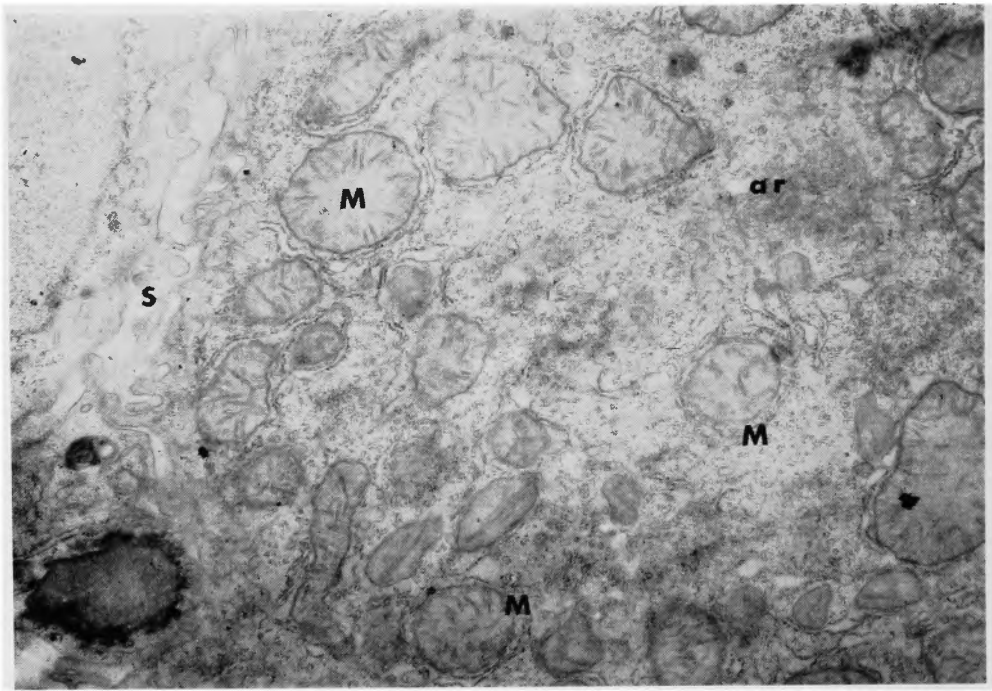


Fig. 13 Note the crenation of mitochondrial membranes and the dispersal of ribosomes in hyaloplasm. 1 hour daily exposure to 100 p. p. m. DCHF B-containing halothane is repeated for 10 days. X 16,000

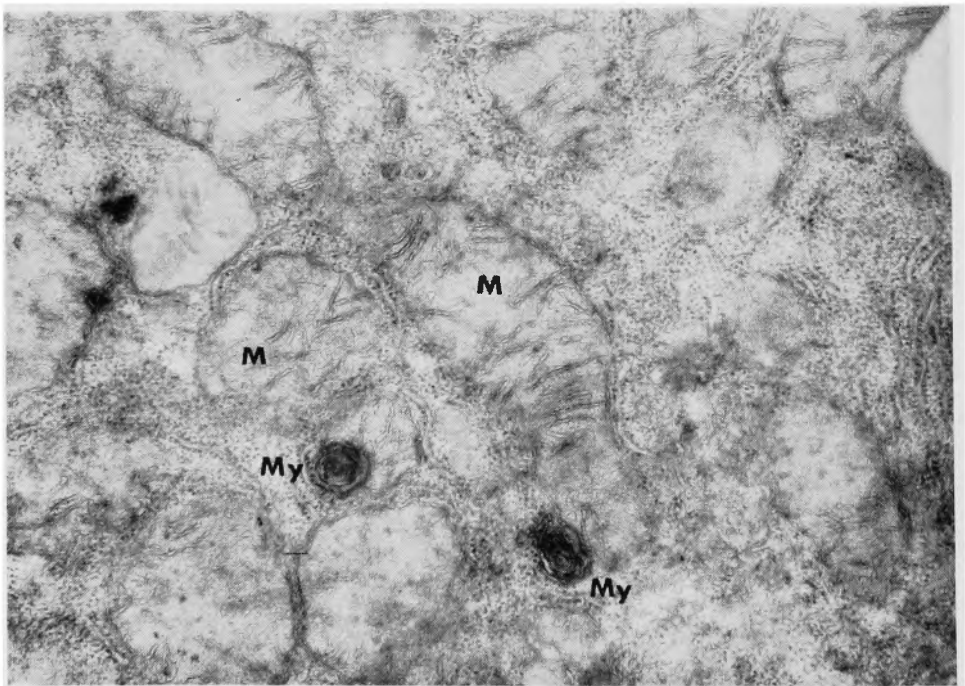


Fig. 14 Portion of the same specimen as Fig. 13. (100 p. p. m. DCHF B) X 27,000

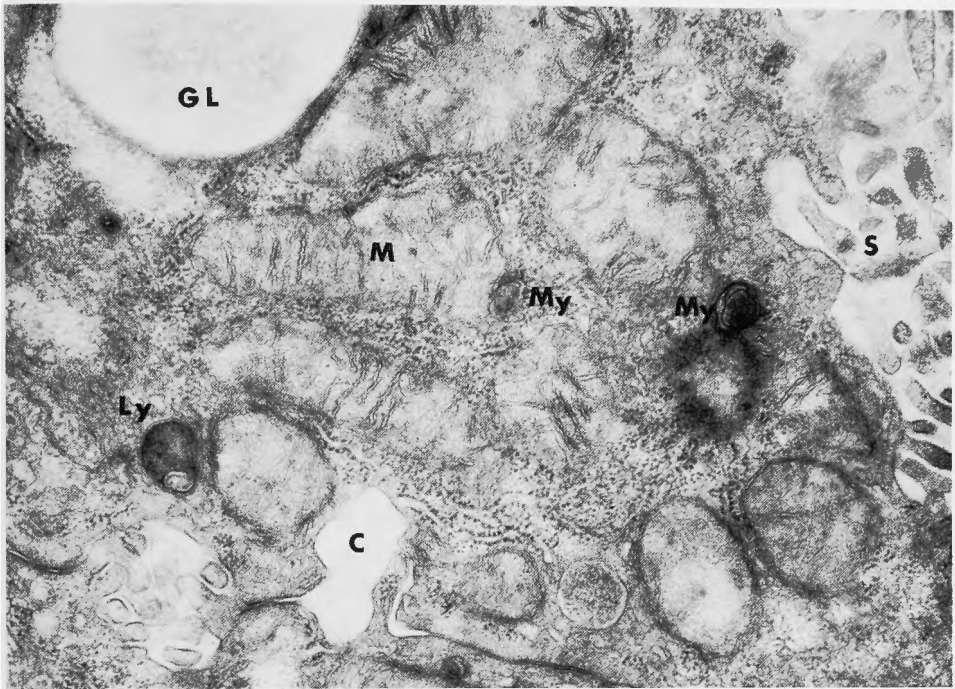


Fig. 15 Myelin figures are attached to mitochondria. Ribosomal dispersion and mitochondrial alteration are observed, and giant liposomes are seen as light holes. X 27,000 (100 p. p. m. DCHFB)

和文抄録

不純物混入ハロセンによる反復麻酔のマウス肝
に及ぼす形態学的変化に関する研究

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ハロセンの発明は麻酔学に画期的な進歩をもたらした。ハロセンは多くの長所を持つものであるが、なかでも肝障害を誘発しないのがその特色の一つとせられてきた。しかしながら、臨床面において極く稀にはあるが、ハロセン麻酔後に猛烈な肝炎を誘発し、肝死より短期間に患者を死に至らしめることがある。しかもこれらの症例は、市販ハロセンにて何回か繰返し麻酔せられたものに多くみられるもので、ハロセン或はその不純物の影響によるものと考えられる。ハロセン不純物にも種々あるが、主なものは DCHFCB と DCHF B の二つである。この研究は、ハロセンにこれらの不純物を少量加えてマウスを反復麻酔し、肝障害について形態学的に追求したものである。

体重 17~22 gr. の D.D 系マウスを円筒形のガラス容器に入れ、1%ハロセンに 50, 100, 250 p.p.m. の不純物を加えた GAS にて、夫々 1 日 1 時間の麻酔をかけることを 10 日間繰返し、気化には純酸素を用いている。マウスは随時に水・標準食を摂れる様にし、環境温度は 24°C とした。10 日目の麻酔終了後に半数のマウスより肝を採り、残りの半数はなお 10 日間 (回復期) 麻酔なしで放置してから同様に標本とした。光顕用標本はハマトキシリン・エオジンとズダン III 染色により、電顕用には固定をオスミウム、包埋をエホン 812 でおこなった。

市販ハロセン、及びこれに 50 p.p.m. の DCHF B などを加えたものでは、グリコーゲンの減少と小葉中心部の著明な脂肪沈着を認めるがこれらは全ての実験群

にみられるもので、他の所見と同様回復期を経たものでは殆んど消退する、また Endoplasmic Reticulum は軽度膨脹して、その近辺に Liposome 及び Giant Liposome の出現をしばしば認める。Microbody は幾分数を増し、Mitochondria は概ね正常に近いが時として奇異な形態を呈することがある。DCHFCB の濃度をあげて 250 p.p.m. にすると、Endoplasmic Reticulum の膨脹は増大し、Liposome・Giant liposome・Lysosome も増加して Mitochondria の形態も様々となる。なかには Crenation を呈するものさえある。

DCHF B の場合には、50 p.p.m. の濃度にてても即にご程度の変化がみられることが多く、その上 Myelin figure の出現をみる標本もある。100 p.p.m. では、変化は一層強調せられており、250 p.p.m. ではマウスは実験に耐えきれない。また、これら不純物は、光顕下の観察にて肝の粟粒え死をおこしていることもあるが DCHFCB と DCHF B とでは違った形をとる。

全実験群を通じて 10 日間の回復期を経たものでは肝細胞は所見に乏しくなり、これらの諸変化は可逆的なものであることを意味する。そして、これらの変化がハロセン不純物に特有なものであるか否かは別として、少なくとも不純物の毒性の結果によるものであることに疑いはない。ハロセン中の不純物は、昨今極めて少なくなったとは言え、市販ハロセンからこれらの不純物を完全に除去することこそ我々にとって重要である。